Preparation of Metronidazole Containing Film Dosage Forms from Sodium Alginate

Abstract

Metronidazole, an antiprotozoal drug, and Sodium Alginate (Alg-Na), a natural polysaccharide, have been used for wound healing by local application. In this study, film dosage forms containing metronidazole were prepared using Alg-Na as the film base, and the disintegration profile of the film and the drug release rate from the form in a small volume of aqueous medium were investigated. The forms were obtained by preparing with either 3-5% low-molecular-weight Alg-Na or 1.5% high-molecular-weight Alg-Na containing 0.1% chitin. Though all the forms immediately swelled in physiological saline, each disintegration profile differed according to the species of Alg-Na or the base concentration. Film disintegration did not affect the drug release rate from the dosage forms and about 80% of metronidazole incorporated in the form were released in all case. These results suggest that these forms can effectively enable drug release on the skin, which is covered by effusion exuded from the wound.

Keywords

Chitin; Drug Release Rate; Film Disintegration; Metronidazole; Sodium Alginate

Abbreviations

MNZ : Metronidazole  
Alg-Na : Sodium Alginate  
FD : Film Dosage Form  
HX : Hydroxylamine  
CMEC : 1-Cyclohexyl-3-(2-Morpholinoethyl) Carbodiimide Metho-P-Toluenesulfonate

Introduction

The antiprotozoal drug Metronidazole (MNZ) has been used for the treatment of Trichomonas or Entamoeba infections. MNZ is also utilized to eradicate Helicobacter pylori, which is associated with gastric or peptic ulcers [1]. However, the appearance of side effects and neurological symptoms, such as peripheral neuropathy, was reported when the drug was orally administered for a long time [2,3]. Local application of MNZ has been used to treat wounds after surgery [4]. Recently, MNZ gel has been adapted for local skin applications to prevent bacterial breeding, which causes the generation of a malodor in patients suffering from ulcerating tumors [5,6]. Addressing material is usually applied at the region of the wound because
of the effusion exuded at the wound. For example, the sodium salt (Alg-Na) of alginic acid, an algal polysaccharide that consists of α-L-guluronic acid and β-D-mannuronic acid is used as a dressing material to ensure wound healing [7,8].

Alg-Na has been widely used as a food additive, a tablet disintegrator, or a gelling agent [9,10]. A thin film can be simply prepared with Alg-Na using the casting method without the need for dissolution in organic solvents or heating [11-13]. Film Dosage forms (FDs) are films that contain therapeutically active compounds. FDs prepared using water-soluble polymers, such as Alg-Na are an attractive option, since they quickly swell in body fluids to release the active compound to the affected site. Therefore, FD is an excellent tool by which drugs can be delivered to local disease sites [14,15]. In the present study, FDs containing MNZ were prepared using Alg-Na as the film base, which was further modified with an additive, such as chitin. The disintegration profile of the film was assessed by measuring the amount of Alg-Na dissolved in each FD and drug release rates from the dosage form in limited aqueous medium.

Materials and Methods

Materials

Three high-molecular-weight polymers of Alg-Na, Alg-A (Nacalai Tesque Inc., 300 cps; Kyoto, Japan), Alg-B (Nacalai Tesque Inc., 500 cps), and Alg-C, (Kibun Food Chemifa Co., 150 M; Tokyo, Japan) and 3 low-molecular-weight polymers of Alg-Na, Alg-D (Kimica Co., IL-1; Tokyo), Alg-E (Kimica Co., IL-6), and Alg-F (Kimica Co., IL-6G) were used as the film base, as shown in table 1. The model drug, MNZ, methylcellulose (4,000), chitin and Hydroxylamine (HX) were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Chitosan (fine powder, degree of deacetylation: 75-85%) was obtained from Kimitsu Chemical Industries Co. Ltd. (Tokyo). A watersoluble carbodiimide, 1-cyclohexyl-3-(2-morpholinoethyl) Carbodiimide Metho-P-Toluenesulfonate (CMEC) was purchased from Aldrich Chemical Co. (Milwaukee, WI, USA). All the other chemicals used were of reagent grade and were obtained from commercial sources.

<table>
<thead>
<tr>
<th>Name of film base</th>
<th>Manufacturer (species)</th>
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<tbody>
<tr>
<td>Alg-A</td>
<td>Nacalai Tesque Inc. (300 cps)</td>
</tr>
<tr>
<td>Alg-B</td>
<td>Nacalai Tesque Inc. (500 cps)</td>
</tr>
<tr>
<td>Alg-C</td>
<td>Kibun Food Chemifa Co. (150 M)</td>
</tr>
<tr>
<td>Alg-D</td>
<td>Kimica Co. (IL-1)</td>
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<tr>
<td>Alg-E</td>
<td>Kimica Co. (IL-6)</td>
</tr>
<tr>
<td>Alg-F</td>
<td>Kimica Co. (IL-6G)</td>
</tr>
</tbody>
</table>

Table 1: Film base for the preparation of FDs.

Methods

FD preparation: FD was prepared as follows: 1.5-5.0% (w/w) Alg-Na or the solution containing 0.1% additive, such as chitin was dispersed in deionized water to prepare the film base solution. MNZ (10 mg) was added to 10 g of the base solution and then the mixture was thoroughly mixed by sonication and poured (3 g each) into individual plastic Petri dishes (diameter, 54 mm). The dishes were kept for 24 h at 37°C, after which circular films formed were transferred into a desiccator.

MNZ assay: MNZ assay as performed using HPLC. The HPLC system (Hitachi Co., Tokyo, Japan) consisted of a pump (L-2130), UV-detector (L-2400), autosampler (L-2200), and chromatograph-integrator (D-2500) connected to a packed column (150 mm x 4.6 mm, Cosmosil 5C18 MS-II, Nacalai Tesque Inc.). The analysis was conducted at ambient temperature using a mobile phase consisting of 10 mM phosphate buffer (pH 5.5) and methanol (7:3) at a flow rate of 1.0 mL/min [16]. The detector wavelength was set at 320 nm.

Film disintegration test: The film was placed in a plastic dish and 10 mL of physiological saline preheated at 37°C was added [17]. The dish was shaken at 300 rpm in an incubator (SI-300; As One Co., Osaka, Japan) set at 37°C. The medium (0.3 mL) was periodically removed using a plastic syringe and filtered through a syringe-driven filter unit (pore size, 0.45 µm). An equal volume (0.3 mL) of physiological saline at 37°C was added to the dish in the incubator to maintain a constant volume. Aliquots (0.2 mL) of the filtered solution were combined with 0.8 mL of ion-exchanged water in test tubes before vortexing. The amount of Alg-Na in each sample solution (1 mL) was measured using the method described below. Each test was performed in triplicates.

Colorimetric assay for alginate: The reagent solutions used were 20 mM HX in ion-exchanged water and 0.1 M CMEC in 2% pyridine-HCl buffer (pH 5.0). Aliquots of HX and CMEC (1 mL each) were added to 1 mL of the sample solution, followed by vortexing. Each mixture was incubated at 40°C for 20 min, after which 20 mM FeCl3 in 0.1 M HCl (3 mL) was added. The absorbance of the solution was measured at 480 nm.
nm in a quartz cell (1 cm light path) using a spectrophotometer (UV-1200; Shimadzu, Kyoto, Japan). Each absorbance value was normalized to that of a blank reagent. For each test, a calibration curve was constructed using a fresh set of Alg-Na standards.

**Drug dissolution test:** The sample solution was obtained by the same method described in the film disintegration test section. Next, 80µL aliquots of the filtered sample solution were placed in micro test tubes (1.5 mL), to which 720µL of methanol was added to precipitate the polysaccharide dissolved from the dosage form. Samples were mixed and centrifuged at 7,700 × g for 5 min (H-1300; Kokusan Co., Saitama, Japan), and the supernatants were injected onto the HPLC column. Each test was performed in triplicates.

**Results and Discussion**

The composition of Alg-Na, especially the molecular weight, affects film formation. Since FDs were prepared by casting method in this study, 1.5-5% Alg-Na was used as a film base solution considering the viscosity of the polysaccharide solution. Addition of drug to the base solution affected film formation. As shown in figure 1, cracks were observed on films prepared with 1.5% Alg-A containing MNZ so that the film base could form thin circular films when drug-free. In addition, FDs were not obtained with either 1.5% Alg-B or Alg-C. On the other hand, circular films were obtained by preparing with 3-5% low-molecular-weight Alg-Na, such as Alg-D. FDs were obtained from 1.5% Alg-A containing 0.1% chitin or chitosan though the form prepared with 1.5% Alg-A containing 0.1% methylcellulose cracked.

FDs prepared with a water-soluble polysaccharide, Alg-Na, immediately swelled and disintegrated in physiological saline at 37°C, resulting in the dissolution of the film base into the solution. Figure 2 shows the disintegration profiles of FDs prepared with low-molecular-weight Alg-Na. For FDs prepared with 3% Alg-D, 58±7% of the incorporated Alg-Na dissolved at 3 min, and the entire amount of the film base dissolved into the test medium at 10 min. In case of FDs prepared with 4% Alg-D or 5% Alg-D, more than 80% of the film base dissolved into the test medium at 10 min. The disintegration profiles of FDs prepared with 4% Alg-E or Alg-F were slower than those for Alg-D, and the amounts of Alg-Na dissolved at 10 min were 69% and 52%, respectively. In the case of FDs prepared with Alg-A containing 0.1% chitin, the amount of Alg-Na dissolved was 42% at 10 min, as shown in figure 3.

![Figure 1: Images of FDs prepared with Alg-Na containing MNZ.](image-url)

(a) 1.5% Alg-A; (b) 1.5% Alg-A containing 0.1% chitin; (c) 1.5% Alg-A containing 0.1% chitosan; (d) 3% Alg-D; (e) 3% Alg-E, and (f) 3% Alg-F.
Figure 2: Dissolution profiles of Alg-Na from FDs prepared using low-molecular-weight Alg-Na. Each result represents the mean and standard deviation of three independent determinations.

Figure 3: Dissolution profiles of Alg-Na from FDs prepared using 1.5% high-molecular-weight Alg-Na containing 0.1% chitin. Each result represents the mean and standard deviation of three independent determinations.
Figure 4 shows the drug release rates from FDs prepared with low-molecular-weight Alg-Na. MNZ incorporated in the FDs was released immediately when the form was brought in contact with the test medium. For example, 1.4 mg of MNZ was released at 1 min from the FD prepared with 4% Alg-D, and >80% of the drug incorporated in the dosage form was released at 5 min. When FDs were prepared with 3% Alg-D or 5% Alg-D, almost the entire amount of the drug incorporated in the FD was released after 5 min. Furthermore, similar drug release rates were obtained in both cases of FDs prepared with 4% Alg-E and Alg-F. A rapid dissolution profile for MNZ was also observed for the FDs prepared with 1.5% high-molecular-weight Alg-Na containing 0.1% chitin, as shown in figure 5. FD disintegration did not affect the release.
rate of the drug from the dosage forms. These results show that MNZ incorporated in FD dissolves quickly without the complete disintegration of the film matrix after FD is set in restricted amounts of media. Finally, the ingredients of FD, which consist of both Alg-Na and additives, such as chitin result in therapeutic action at the application site [18,19].

Conclusion

Application of MNZ to the local site is a useful therapy for fungating wounds. In this study, FDs containing MNZ were prepared with Alg-Na as a film base, and the disintegration profile of the dosage form and the rate of MNZ release were investigated in a small amount of aqueous medium. FD readily swelled and immediately released MNZ incorporated in the form. This result suggests that the FD prepared from Alg-Na can be a promising candidate as a dosage form containing MNZ since it can facilitate the drug release on the skin that generally gets covered with effusion exuded at the wound.

References